Dissecting the mechanisms of Notch/Akt-induced tumorigenesis: the role of energetic stress and metabolic reprogramming

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Abstract

The simultaneous aberrant activation of Notch and PI3K/Pten/Akt signaling pathways generates massive tumors in flies and is also causative of several aggressive forms of human cancers such as T-cell lymphoblastic leukaemia. However, the mechanism behind this oncogenic cooperation is poorly understood. Here, we used Drosophila to investigate molecular aspects of this interaction using a model entailing the simultaneous over-activation of both Notch and Akt signaling pathways (N^+Akt^+) in the developing fly eye. By employing phospho-proteomic analysis on N^+Akt^+ induced tumors we have identified a specific downstream target belonging to the mitochondrial electron transport chain (ETC). Genetic inactivation of ETC components induces the generation of reactive oxygen species (ROS), which in turn cooperates with Notch signal to fuel tumorigenesis. We investigated the role of stress-activated JNK signaling pathway and sima/HIF1α transcriptional factor in response to high ROS levels. Interestingly, N^+Akt^+ tumors are JNK independent, but rely on HIF1α, the most important driver of glycolytic phenotype in cancer. Furthermore, we found that N^+Akt^+ combination provokes an interorgan glycolytic reprogramming that may underlie tumor growth.

1. Phospho-proteomic assay reveals a role for ATPsynb in Notch-Akt tumorigenesis

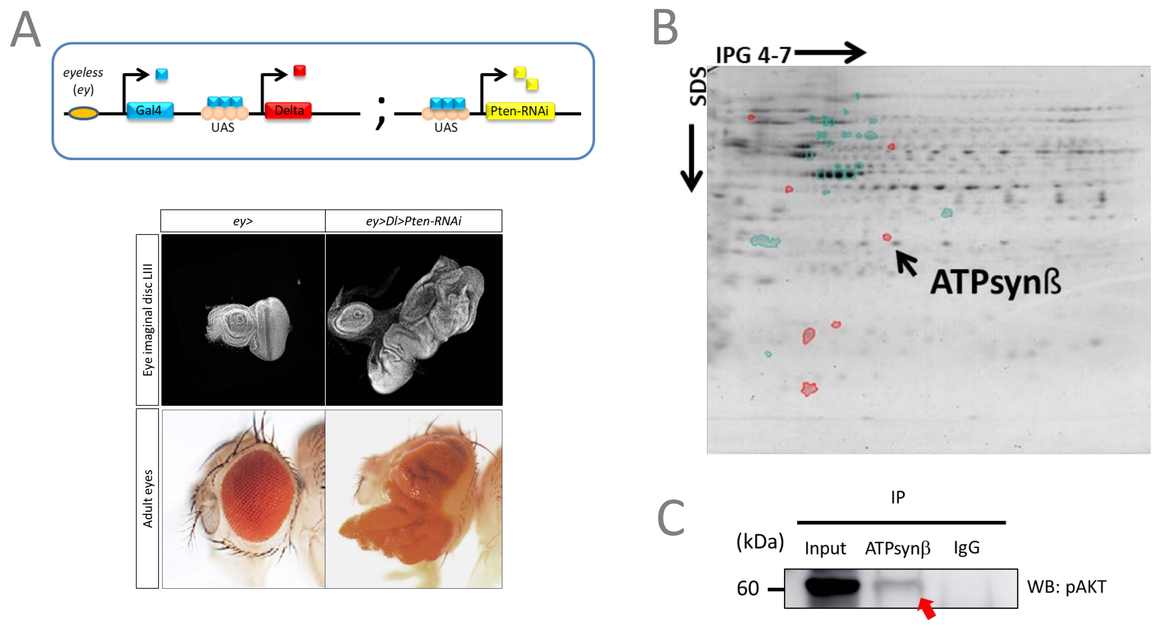
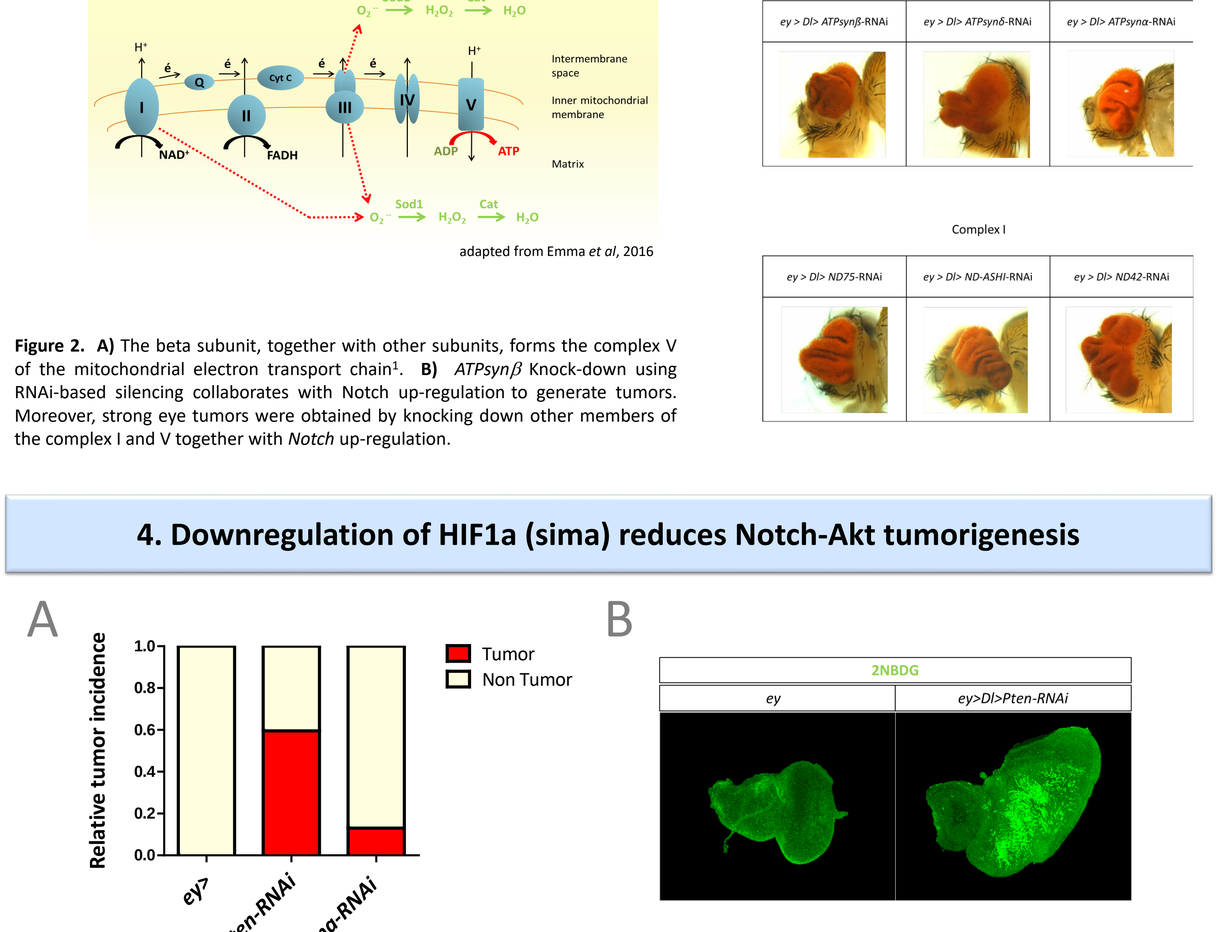


Figure 1. A) Hyperactivation of Notch and Akt pathways in the eye imaginal disc was induced by the overexpression of the Notch ligand *Delta* (*Dl*) and the knock-down of the *Akt* negative regulator *Pten* by interference RNA. These two signals are simultaneously overexpressed under the control of the *eyeless* (*ey*) promoter. **B)** Overlapped 2D-gels. Red dots represent proteins highly phosphorylated in the Notch-Akt condition. Significantly differential phosphorylated protein were selected for mass spectrometry analysis. The top ranked protein was the ATP synthase beta subunit **(ATPsynβ)**, a member of the mitochondrial Complex V. **C)** Co-immunoprecipitation assay reveals physical interaction between Akt and ATPsynβ protein.

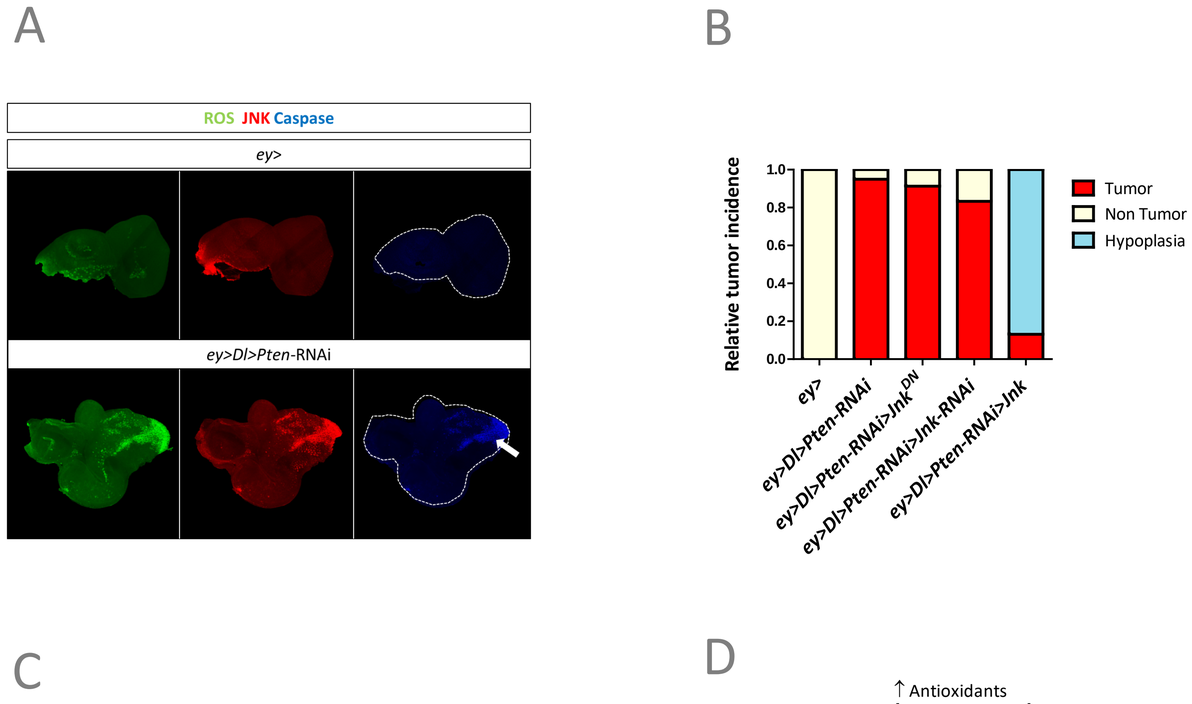
2. Mitochondrial dysfunction cooperates with Notch to promote tumorigenesis, independently of Akt activation



adapted from Emma *et al*, 2016

Figure 2. A) The beta subunit, together with other subunits, forms the complex V of the mitochondrial electron transport chain[1]. **B)** *ATPsynβ* Knock-down using RNAi-based silencing collaborates with Notch up-regulation to generate tumors. Moreover, strong eye tumors were obtained by knocking down other members of the complex I and V together with *Notch* up-regulation.

3. Notch-AKT combination triggers ROS production and activation of JNK stress signaling cascade



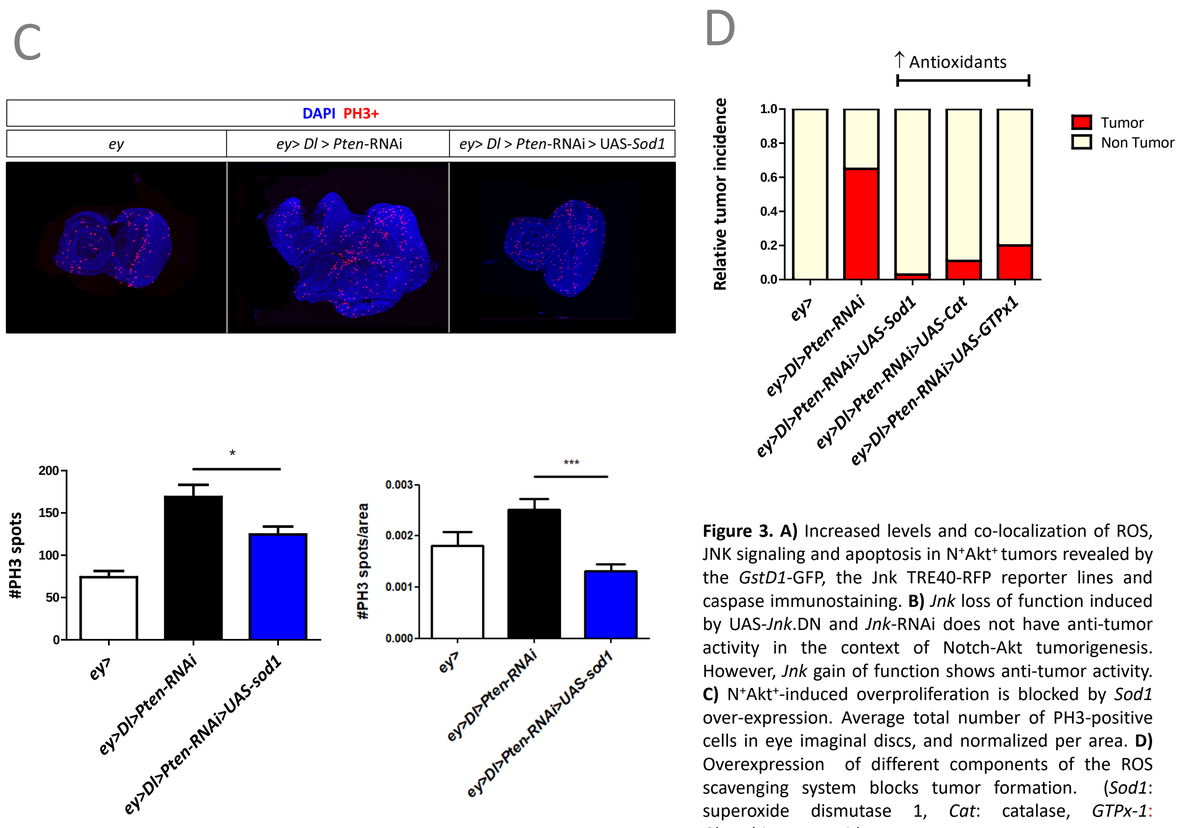


Figure 3. A) Increased levels and co-localization of ROS, JNK signaling and apoptosis in N^+Akt^+ tumors revealed by the *GstD1*-GFP, the Jnk TRE40-RFP reporter lines and caspase immunostaining. **B)** *Jnk* loss of function induced by UAS-*Jnk*.DN and *Jnk*-RNAi does not have anti-tumor activity in the context of Notch-Akt tumorigenesis. However, *Jnk* gain of function shows anti-tumor activity. **C)** N^+Akt^+-induced overproliferation is blocked by *Sod1* over-expression. Average total number of PH3-positive cells in eye imaginal discs, and normalized per area. **D)** Overexpression of different components of the ROS scavenging system blocks tumor formation. (*Sod1*: superoxide dismutase 1, *Cat*: catalase, *GTPx-1*: Glutathione peroxidase.

4. Downregulation of HIF1a (sima) reduces Notch-Akt tumorigenesis

Figure 4. A) Downregulation of *sima* using RNAi reduces Notch-Akt tumorigenesis. **B)** Monitorization of glucose uptake in live tissue using 2NBDG (fluorescent glucose analogue) revealed increased levels of glucose incorporation in N^+Akt^+ tumors.

5. Notch-Akt results in interorgan glycolysis reprograming

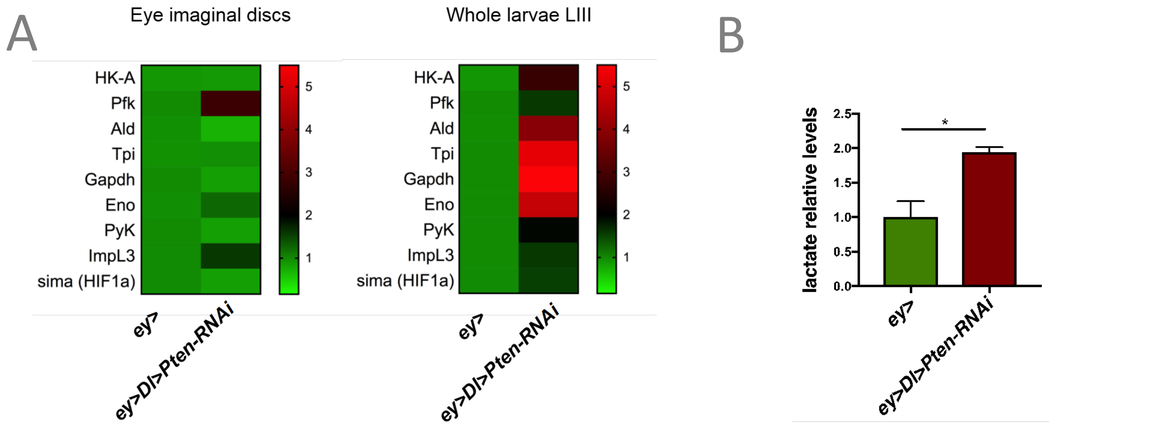


Figure 5. Glycolytic pathway is upregulated in animals bearing N^+Akt^+ tumors. **A)** Heat map of transcriptional levels of several glycolytic enzymes measured by qPCR in eye imaginal discs and third instar larvae. **B)** Measurement of lactate levels by Gas Chromatography-Mass Spectrometry (GC-MS) in whole larvae.

6. LC-MS reveals differences in the metabolome of flies bearing Notch-Akt tumors

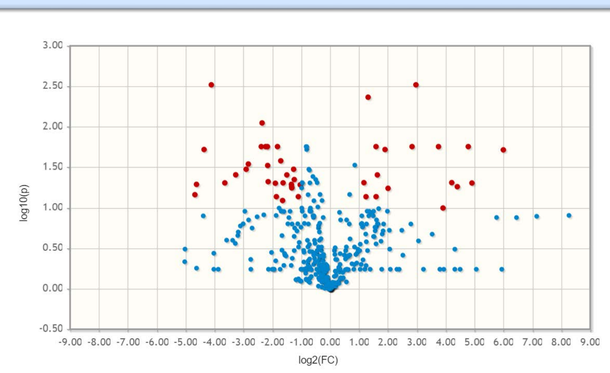


Figure 6. Volcano plot of the metabolites that significatively change in whole larvae after performing large-scale metabolomics analysis by Liquid Chromatography coupled to Mass Spectrometry (LC-MS). Fold change: Tumor-bearing larvae/wild type.

Discussion and future perspectives

In this study we show that Akt activation fuels Notch tumorigenic potential by generating a mitochondrial dysfunction through the phosphorylation of ATPsynβ. Reducing high levels of ROS generated by the mitochondrial dysfunction or the downstream activated sima/HIF1α blocks Notch-Akt tumors. It has been previously proposed that increased ROS levels can induce tumorigenesis by activating the JNK signal transduction pathway2,3,4,5, a classical cell stress mediator6. However, we show that *Jnk* up-regulation reduced tumorigenic potential, indicating that Jnk signal may act as a tumor suppressor in the context of Notch-Akt combination. On the other hand, high ROS levels can stabilize HIF1α protein, a transcriptional factor that plays a central role in the cellular adaptive response to hypoxic conditions and control glycolytic genes expression7. This metabolic switch demands higher amounts of glucose uptake, as happens in Notch-Akt tumors. Glycolytic enzymes however, are not increased at transcriptional level in tumors, but in the whole larvae. Either targeted and large-scale metabolomics revealed significant differences in the metabolome of those larvae with tumors, indicating a possible crosstalk between the tumor and another tissue. Such an interesting possibility, opens new route for therapeutically approaches.

References

1 . Emma, F. *et al* (2016). **2.** Dhanasekaran, D. and Reddy, P. (2008). **3.** Chambers, J.W. and LoGrasso, P.V. (2011). **4.** Ohsawa, S. *et al* (2012). **5.** Wang ,CW. *et al* (2016). **6.** Rios-Barrera, D. and Riesgo-Escovar, J.R. (2013). **7 .** Jung, S.N. *et al* (2018).

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